Analytical Performance At Low Levels and Near the Cutoff

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Medical Laboratory Test

Analytical performance (measuring device)

Clinical performance (related to the claim)

CLSI documents are major sources of terminology, study design, and statistical analysis

What we discussed at this talk

- Performance of a medical laboratory test at low levels is characterized by
  Limit of Blank (LoB),
  Limit of Detection (LoD),
  Limit of Quantitation (LoQ),
  Analytical sensitivity.

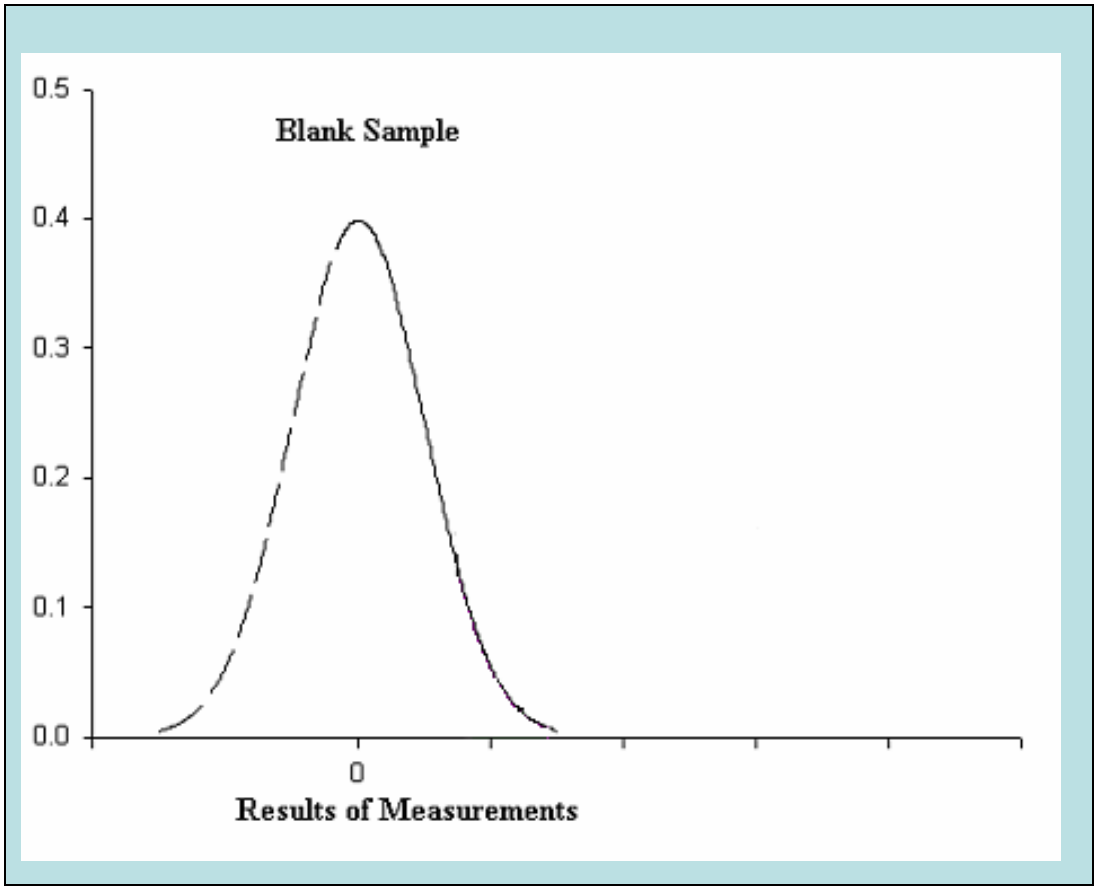
- Confusing terminology

Sometimes, the limit of detection is estimated only on the basis of repeated measurements of a blank sample (20 measurements of zero calibrator) and reported as the mean plus 2 SD of the blank measurements
(THIS IS NOT CORRECT).

We consider quantitative tests;
Similar concept are considered for qualitative tests (underlying continuous signal)

Analytical Limits at Low Levels: Limit of Blank

Blank samples – patient samples with zero concentration of analyte;



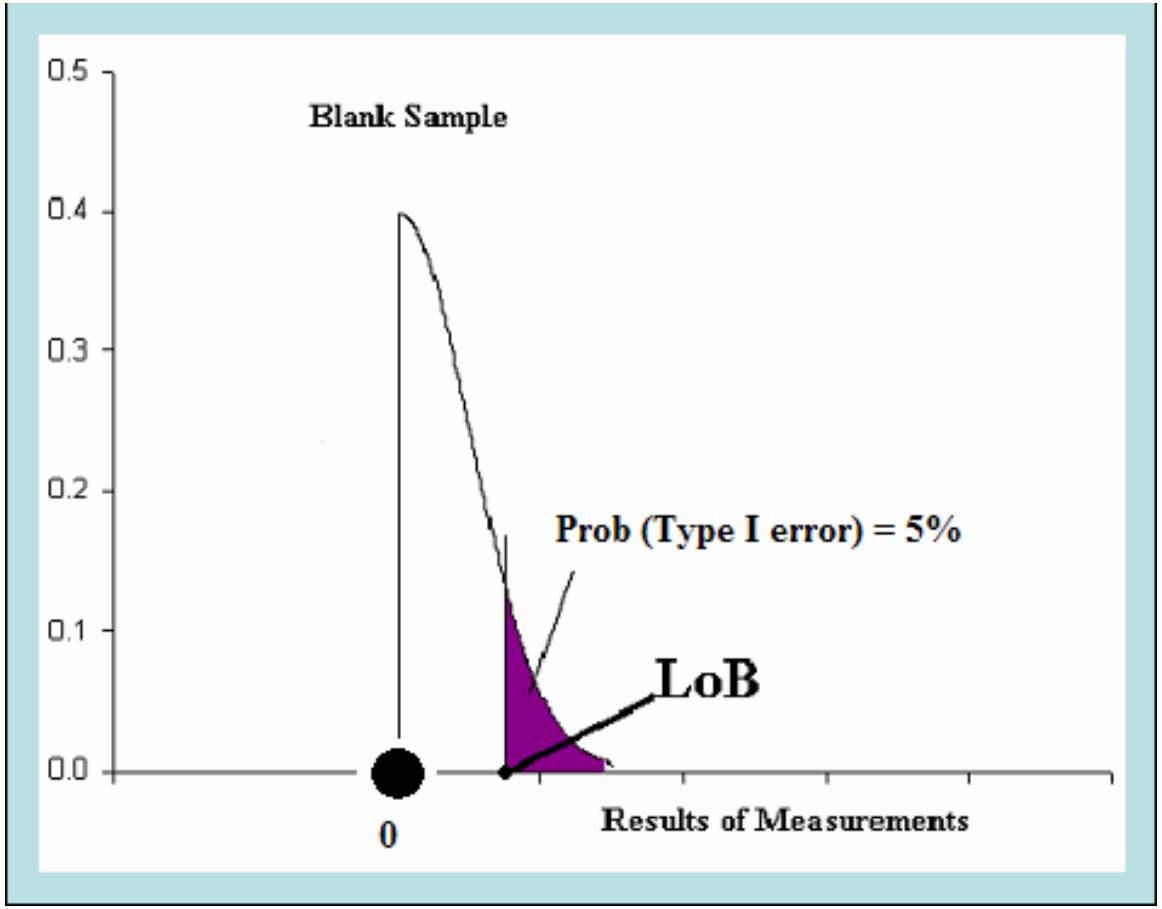
Measurement of Blank samples

Normal distribution

- Fundamental aspect: **Distinguish <u>"Result of measurement, device output"</u> vs <u>actual (true) concentration</u>**
- Assume that there is no systematic bias (traceability).

Limit of Blank

In the clinical practice, the tests report only non-negative values (truncated at zero and thus non-normal).

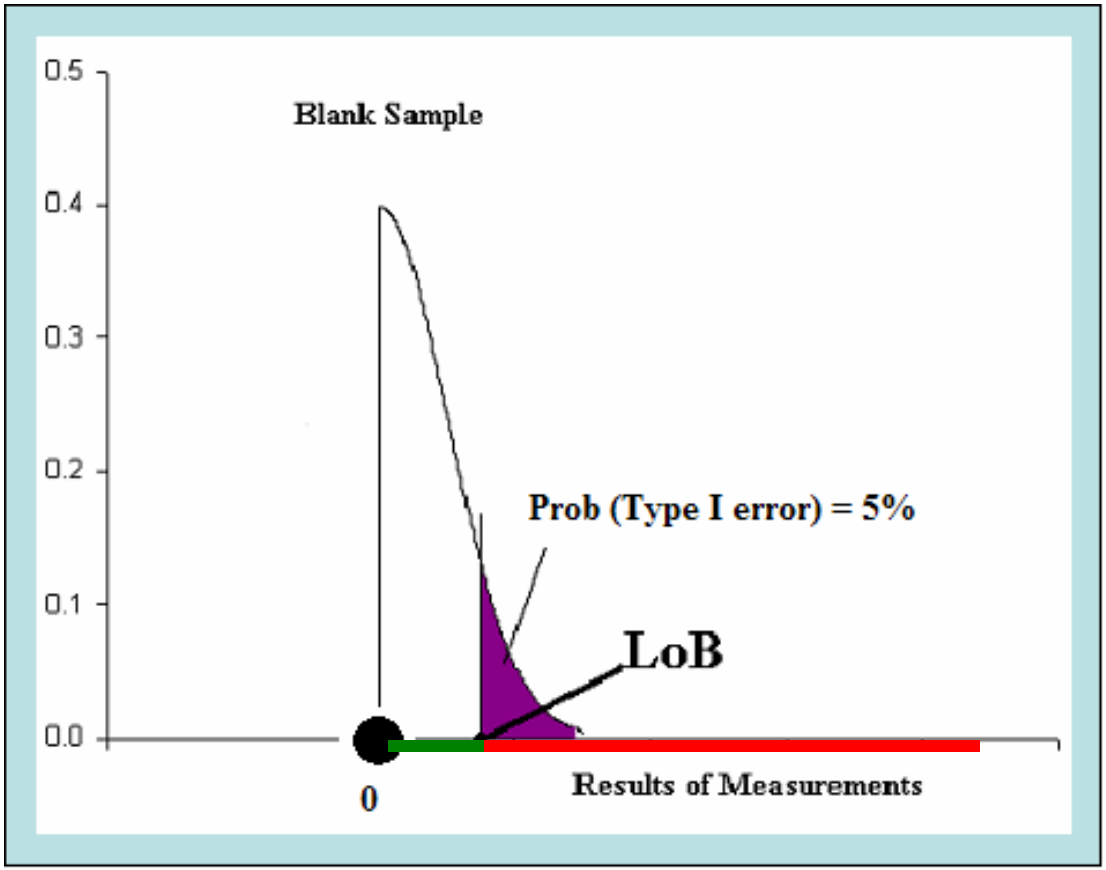


$\alpha = 5\%$

LoB = 95th percentile of all measurements of blank samples

Limit of Blank

Limit of Blank –a limit that is only exceeded with a probability of α for a Blank sample measurement.



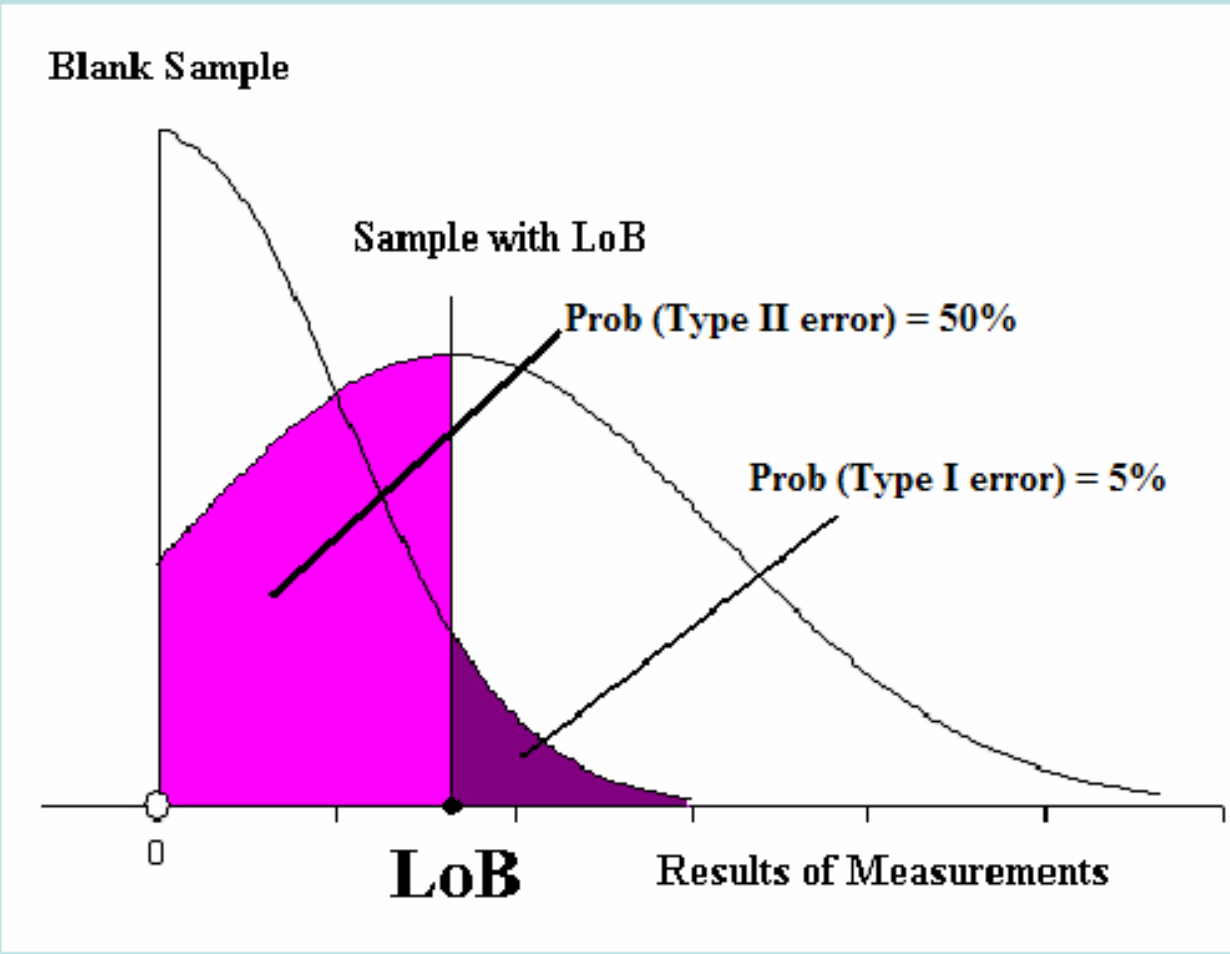
Measurement result ≤ LoB → "Analyte Not Detected"

Measurement result > LoB → "Analyte Detected"

Limit of Detection (LoD):

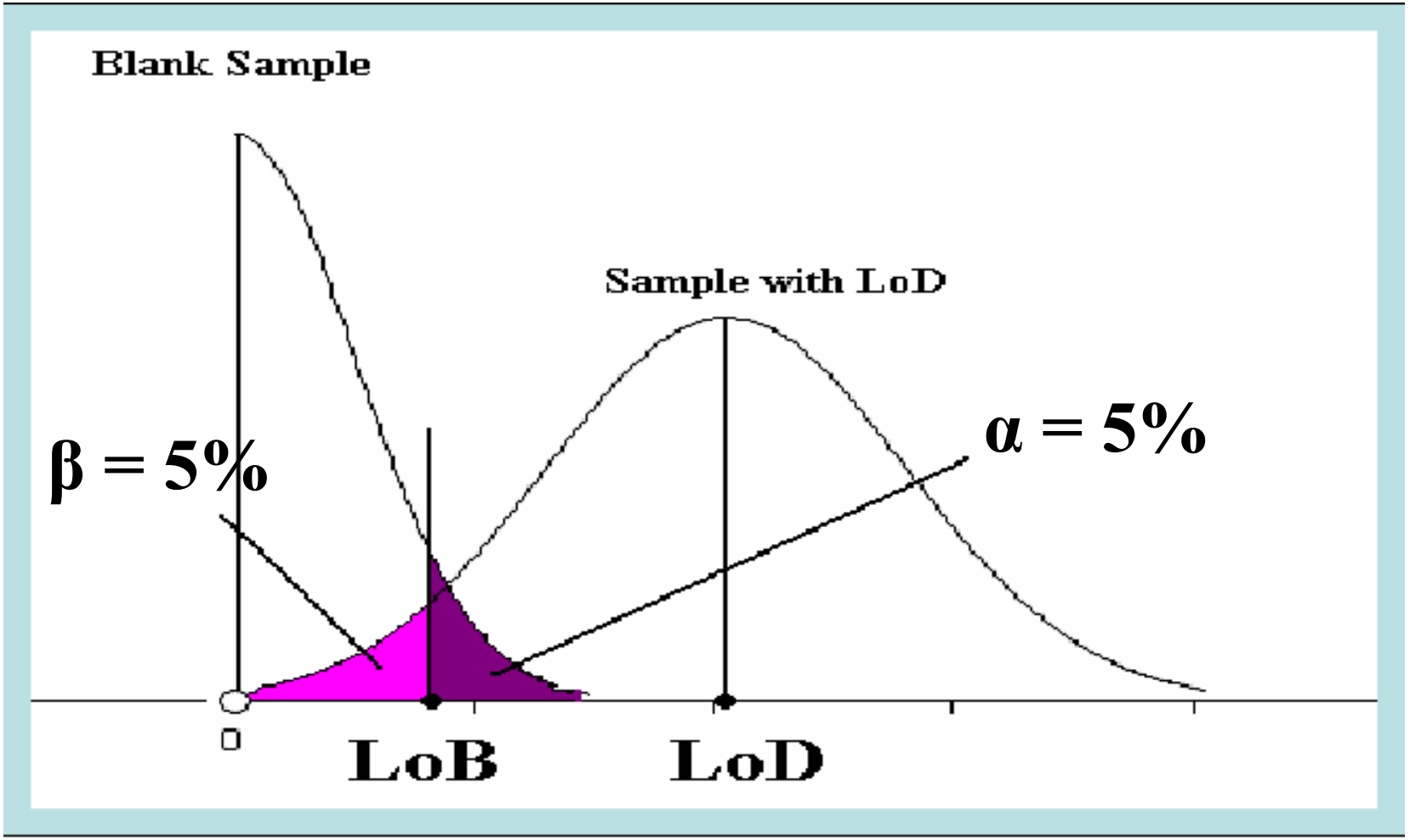
The lowest actual amount of analyte in a sample that can be <u>detected</u> with stated probability (usually, 95%).

The amount of analyte of LoB <u>is not</u> the LoD (**LoB < LoD**).



A sample with a true concentration exactly at LoB will have **only a 50%** chance of returning a value that is interpreted as showing the presence of the analyte.

With a sample having the actual concentration of LoD, only β of the measurements are erroneously declared not different from the blank.



Limit of Blank and Limit of Detection

Limit of Blank (LoB):

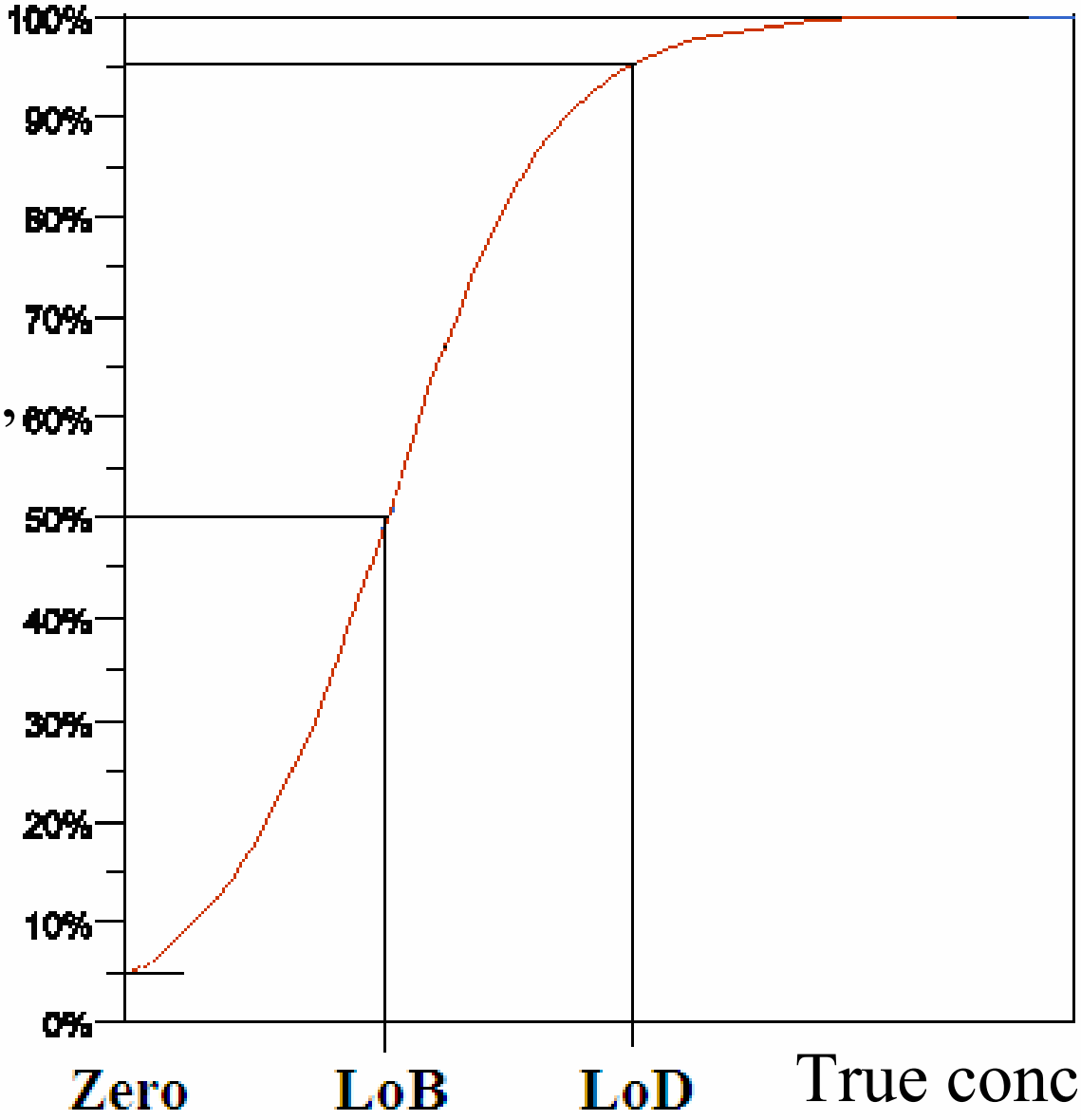
a limit that is only exceeded with a probability of 5% ($\alpha = 5\%$) for a blank sample measurement;

an actual (true) concentration in a sample that is detected 50% of the time and is not detected 50% of the time;

Limit of Detection (LoD):

an actual (true) concentration in a sample that is detected 95% ($\beta = 5\%$) of the time.

Percent of "Detected" results



Sources of variability:

- **Blank samples**
  - Blank samples are the samples which are identical, in principle, to the samples of interest except that the analyte is absent. For example, blank might be samples that are stripped of the component, samples from non-diseased subjects for tumor marker,…
  - Type of the matrix is important.

Sources of variability (cont.):

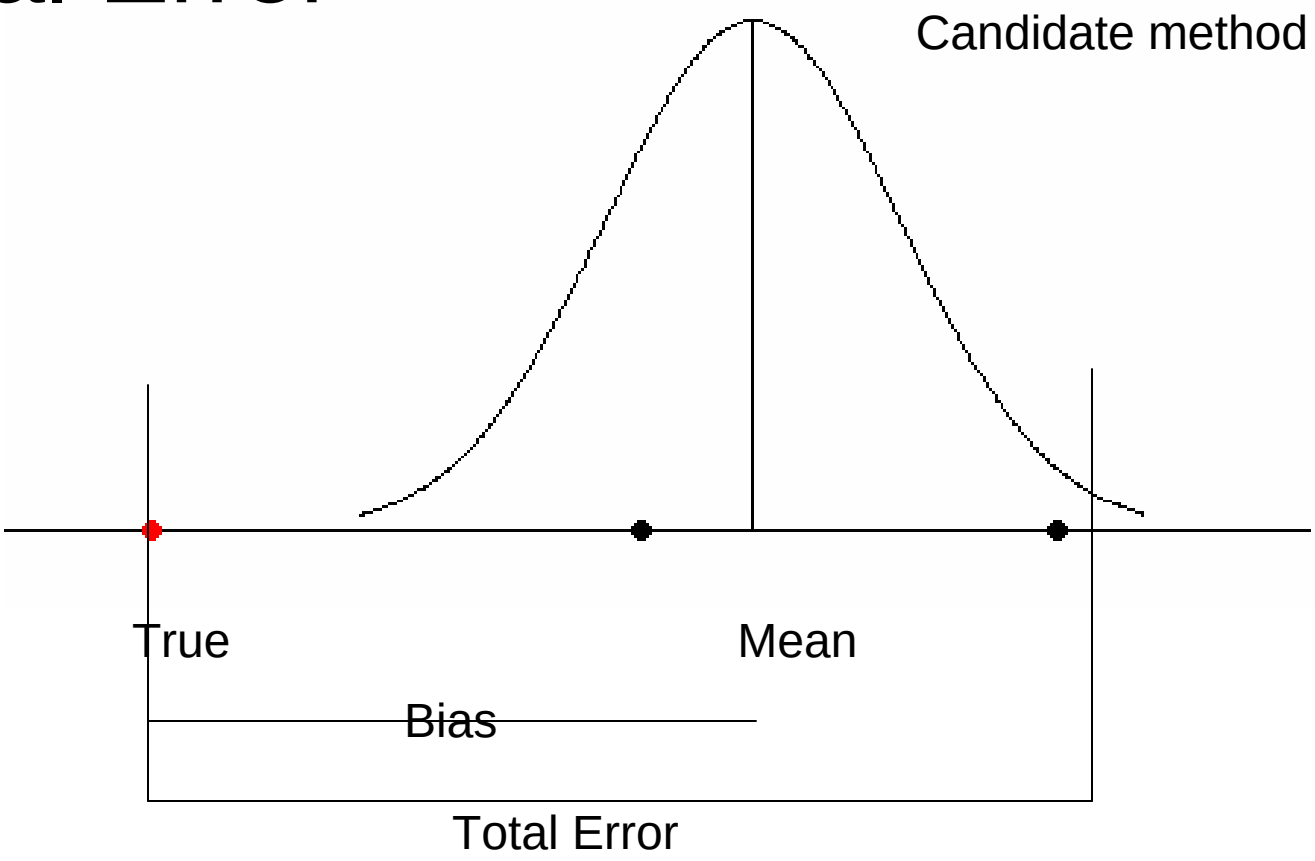
- Blank samples must be similar to patient samples.
  It is preferred to compile measurements from a number of patient samples
  (**CLSI EP17-A**, at least 60 blank measurements of at least 5 samples).
  Zero calibrator may be not appropriate.

- **Within-laboratory precision**: measurements should be spread over several days including different operators.
  Consider at least 2 reagent lots
  (LoD is max among two lots).

Limit of Quantitation (LoQ)

The lowest amount of an analyte in a sample that can be reliably detected *and* at which the total error meets the laboratory's requirements for accuracy.

Total Error



Total Error= TE = |Bias| + 2*SD

Meaning: 95% of the time, the true value is not more than TE from the observed result

Limit of Quantitation (LoQ)

The lowest amount of an analyte in a sample that can be reliably detected *and* at which the total error meets the laboratory's requirements for accuracy.

Depending on the defined goal for error, the LoQ could be equal to the LoD or it could be much higher. It could not be lower than LoD.

$$LoB < LoD \leq LoQ$$

Example

Assay for the quantitation of hepatitis C viral (HCV) RNA in the serum or plasma.

Limit of Blank (α=1%, samples from the negative patients, blood donors)

3,100 copies/mL

Limit of Detection (β = 5%)

5,500 copies/mL

Limit of Quantitation (requirement for accuracy were %CV$\leq$ 20%, syst. bias $\leq$2%, total error $\leq$ 50%

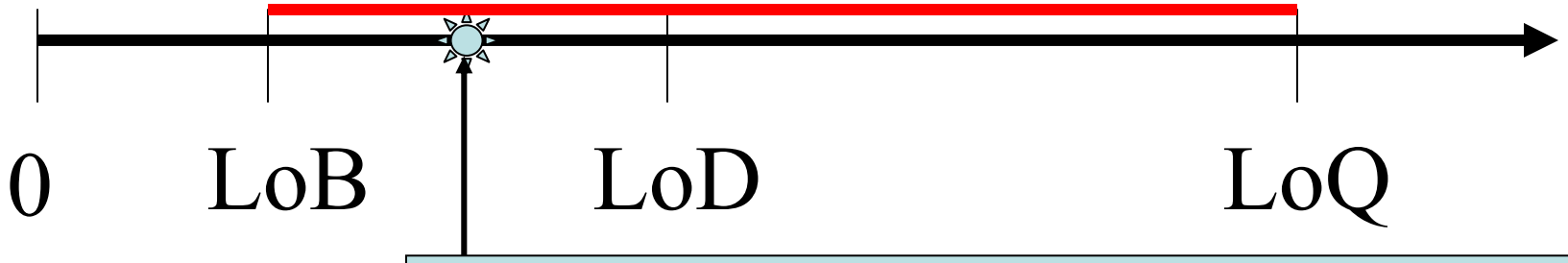
9,000 copies/mL

Reporting Intervals for Quantitative Results

Reports of quantitative values should depend on where the observed result lies relative to the analytical limits at low levels.

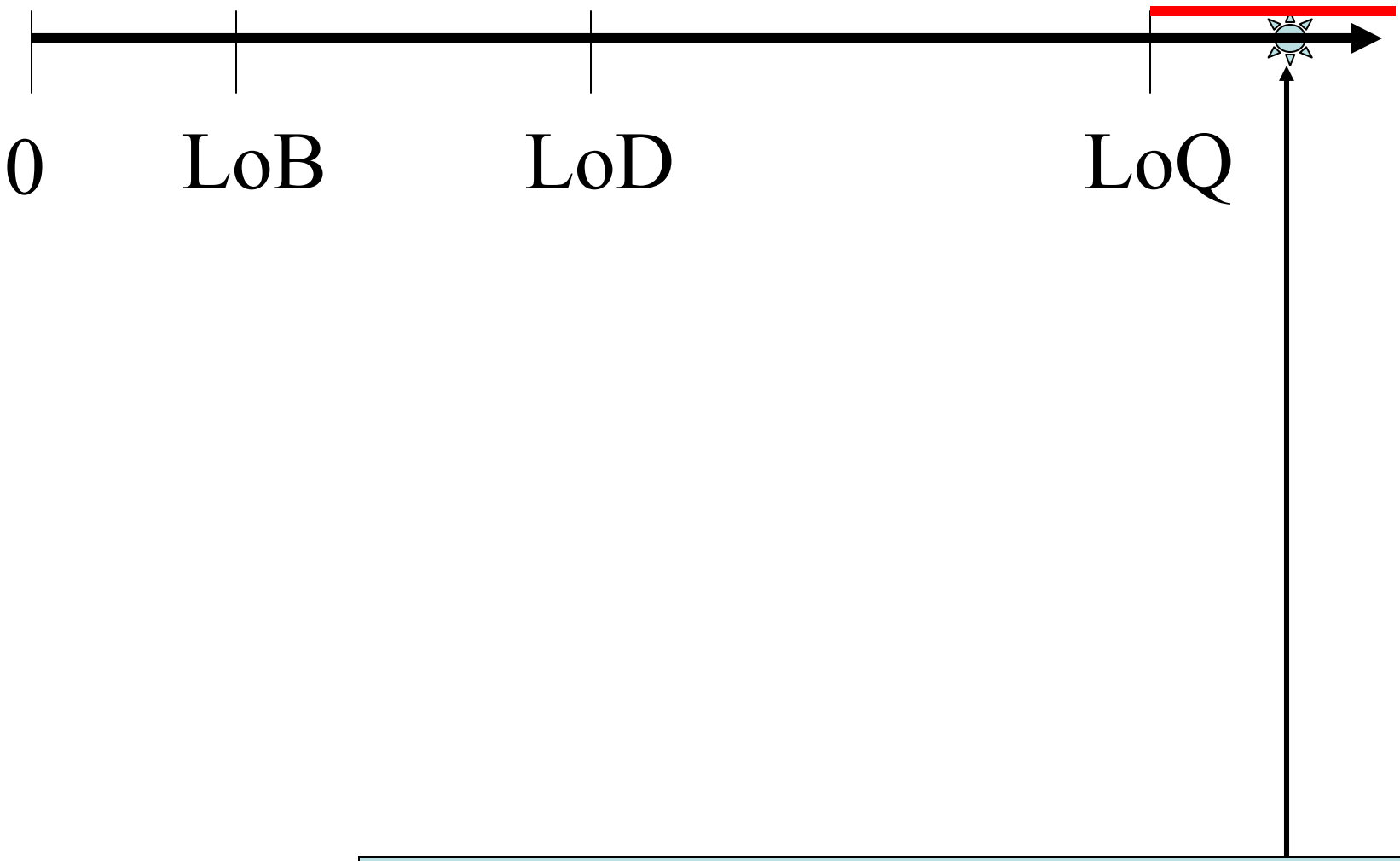
Result ≤ LoB

Report
"Analyte
not detected"



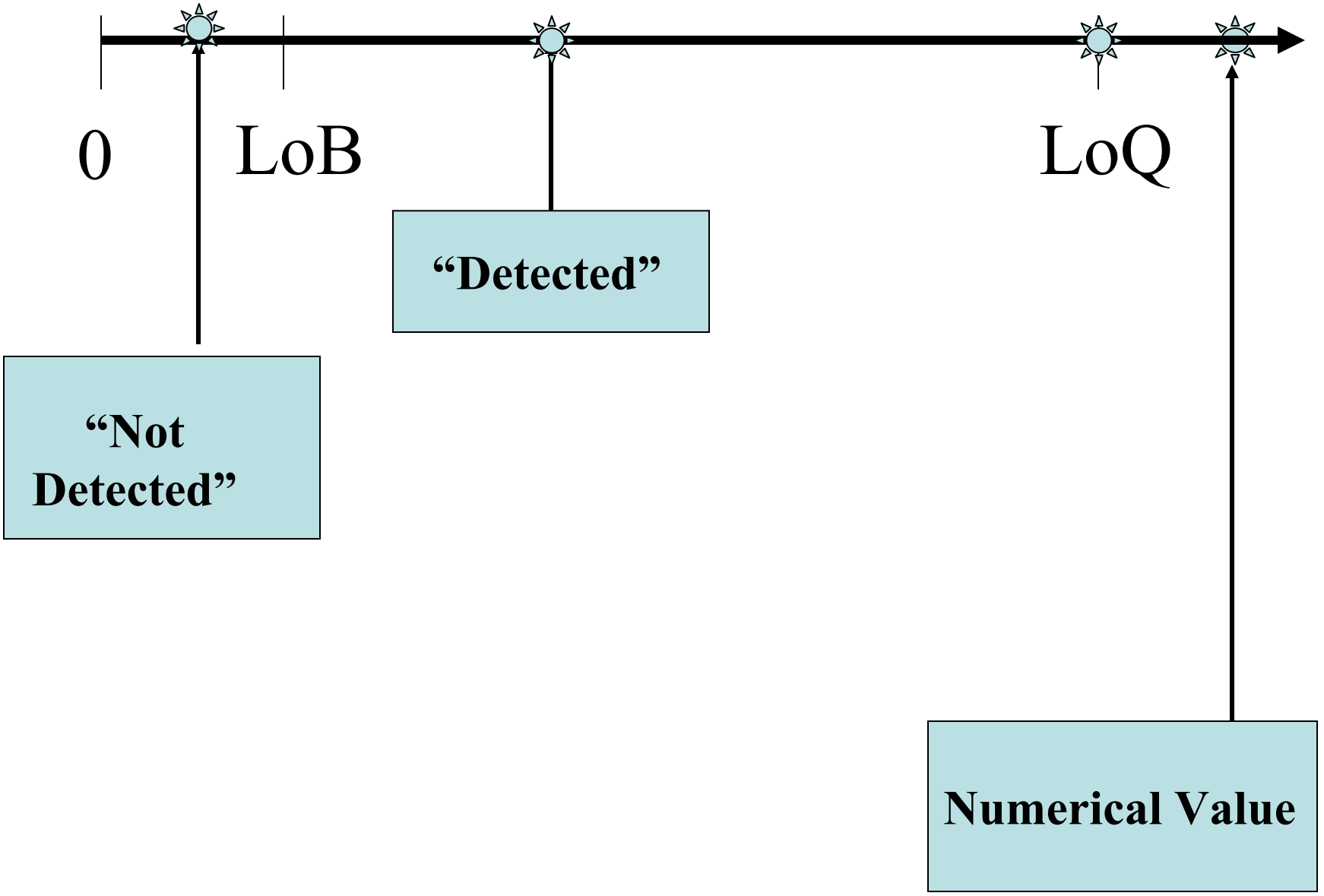
LoB < Result ≤ LoQ

Report "Analyte detected"

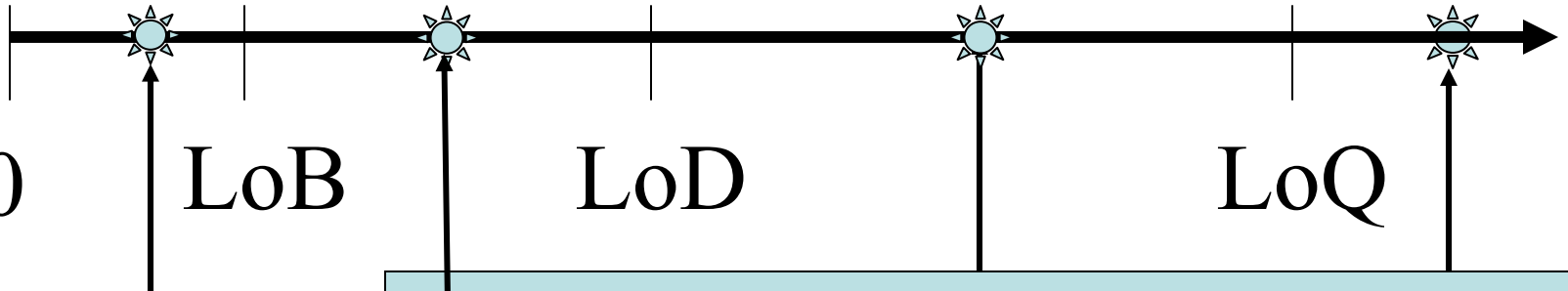


Result ≥ LoQ

Report the result as measured


0
LoB
LoQ
"Detected"
"Not Detected"
Numerical Value

Interpretation



Result ≤ LoB

Interpretation "Analyte is absent OR present but concentration < LoD"

Interpretation "Analyte present; cannot be quantified; detection is unreliable"

Interpretation "Analyte present; cannot be quantified; detection is reliable"

Interpretation, numerical value (look at bias and precision)

Notes: WRONG interpretations: Zero, <LoB

Terminology

ISO	CLSI	Other terms
Critical Value	**Limit of Blank**	Cutoff for signal detection; Detection cutoff; Decision limit; Limit of absence
Limit of Detection	**Limit of Detection**	Labeling of devices: sometimes, it is not provided
Limit of quantitation	**Limit of Quantitation**	Determination limit; Functional sensitivity

Analytical Sensitivity

The limit of detection should not be confused with the analytical sensitivity.

Two different meanings:

1) Analytical sensitivity as an umbrella concept related to all performance characteristics at low levels (not accepted by ISO)

2) ISO definition – slope of the calibration curve

Analytical sensitivity (as a slope of calibration curve) was introduced in 1912 in clinical chemistry; widely used in physics, engineering.

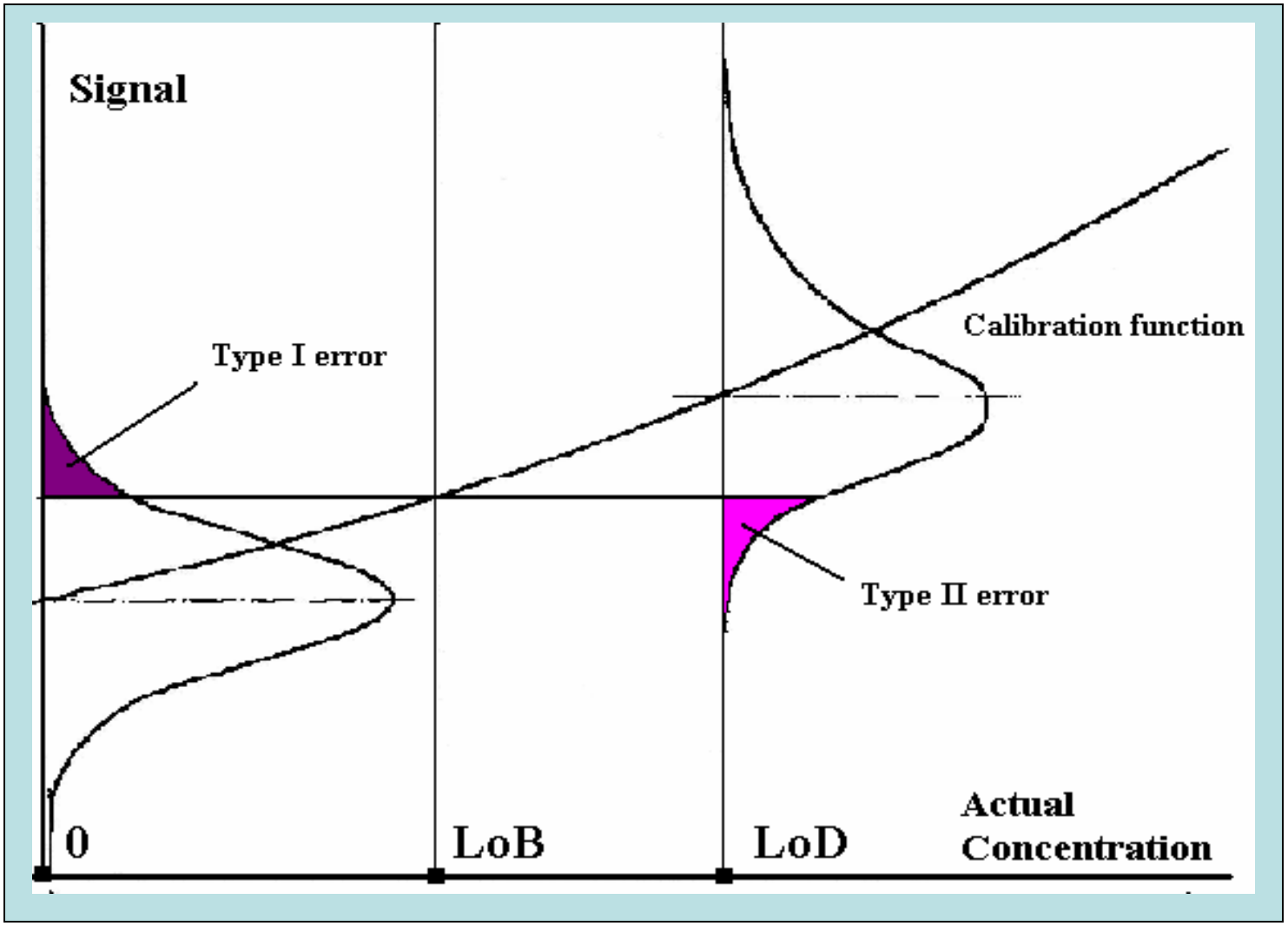
Analytical Sensitivity

According to ISO,
"analytical sensitivity is the change in response of a measuring system divided by the corresponding change in stimulus."

Oxford English Dictionary: " sensitive – indicating slight changes of condition, easily affected by the external forces…"

Fundamental concept:
ability to respond to slight <u>changes</u>

The calibration function relates the mean of the measured response to the actual concentrations.



Analytical sensitivity is not the same as the limit of detection.

- Even if the slope of the calibration function is steep, the LoD can be large if there is a high variation of the measured response.

- On the other hand, a method with a moderate slope of the calibration function and very low random variation of the response can have a low LoD.

Analytical sensitivity (as a slope of the calibration curve) may not be practical

- It is impossible to compare analytical sensitivities of two methods that rely on different response variables;

- Modern instruments provide a direct numerical read- out of the measured quantity and the user may be not interested in the instrument internal operation.

Limit of detection is more relevant.

Example of Labeling (NOT Appropriate):

Analytical sensitivity (limit of detection) is the lowest concentration of the analyte ABC that can be detected with 95% probability. It was estimated as the mean value of zero calibrator plus 2 times standard deviation of 20 measurements. The limit of detection was XYZ.

"Truth" in Labeling

Analytical performance at low levels:
Limit of blank for the zero calibrator based on 20 measurements was XYZ with type I error of 2.5%.

Problems: LoB may be biased. No estimation of LoD.

I) Clinical cutoff is higher than the analytical cutoff (LoB)

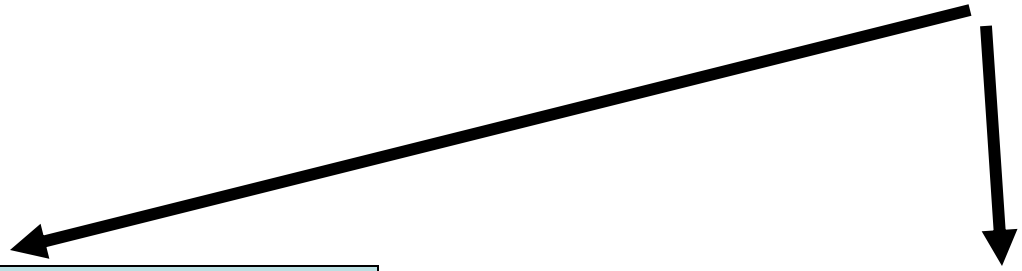
(Non-disease subjects have some amount of analyte)

Cutoff =C

II) Clinical cutoff is the analytical cutoff (LoB)

(no analyte vs analyte present)

Cutoff =LoB



II.1) LoB >0

Samples with zero concentration have noisy results

II.2) LoB=0

For example, ultrasensitive assay

Samples with zero concentration have zero results

Analytical Sensitivity vs Clinical Sensitivity

Analytical sensitivity is a change in the signal divided by the corresponding change in the concentration (slope of calibration curve).
Or all performance characteristics related to the low levels.

Clinical sensitivity is the proportion of patients with a well-defined clinical disorder whose test values are positive. Clinical sensitivity = TP/(TP+FN)

Summary

- The analytical performance characteristics of a medical test at low levels are LoB, LoD, and LoQ.
- Analytical sensitivity is not the same as LoD.

"Define words correctly and you will free the world of half of problems"

Rene Descartes,
French philosopher and mathematician

References

1. **CLSI document EP17-A (2004):** ***Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline***
2. **CLSI document EP12-A2 (2008):** ***User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition***

3. Currie AL, et al. Nomenclature in evaluation of analytical methods including detection and quantification capabilities (IUPAC Recommendations). *Pure Appl Chem.* 1995;67:1699-1723.
5. Brown E.N., McDermont T.J., Bloch K.J., McCollom A.D. (1996). Defining the smallest analyte concentration an immunoassay can measure. *Clinical Chemistry*. 42: 893-903.
6. Linnet K, Kondratovich M. (2004). Partly nonparametric approach for determining the limit of detection. *Clinical Chemistry*. 50: 732-740.

Thank you very much !!!

Questions??

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